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Demonstration of Cord Formation by Rough *Mycobacterium abscessus* Variants: Implications for the Clinical Microbiology Laboratory▽†

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In low-income countries some infections caused by nontuberculous mycobacteria are misdiagnosed as multidrug-resistant tuberculosis. In most of these settings the observation of microscopic cords is the only technique used to identify *Mycobacterium tuberculosis* in the laboratory. In this article we definitively demonstrate that *Mycobacterium abscessus*, an emerging pulmonary pathogen, also forms microscopic cords.

Mycobacterium tuberculosis complex (MTC) species, when grown in a liquid medium without detergent, form cords in tight bundles, consisting of bacilli aligned in parallel, end to end, and side to side, along the long axis of the cord (see reference 6 for a recent review). *M. tuberculosis* microscopic cords were first observed by Robert Koch in 1882, but their significance increased in 1947, when studies performed by Middlebrook et al. linked this phenotypic characteristic to the virulence of MTC organisms (16). From these pioneering studies to now, cord morphology has been considered to be a distinctive feature of the MTC, and its detection, in smears from liquid cultures, is a reliable criterion for the rapid presumptive identification of MTC isolates in many laboratories around the world (6, 12, 14, 20, 23). The first demonstration that cord formation could occur in other mycobacterial species was in 2008, when microscopic cords in a human clinical isolate of *Mycobacterium marinum* were described (21).

In 2010 we demonstrated that *M. marinum* produced true cords (bacilli arranged in parallel along the long axis of the cord) and not clumps (aggregates of bacilli in a random orientation), obtaining the first scanning electron microscopy (SEM) images of the mycobacterial cord ultrastructure (11). In the same work we demonstrated that the phenomenon of cording was also present in rough variants of other nontuberculous mycobacteria (NTM) such as *Mycobacterium vaccae*, *Mycobacterium gilvum*, *Mycobacterium obuense*, *Mycobacterium chubuense*, and *Mycobacterium parafortuitum* (11).

The study of the cording phenomenon by SEM aims to eliminate any doubt regarding the ability of NTM to form cords. This is necessary because the use of cording as a criterion for the identification of MTC isolates from clinical cultures has been adopted as a standard methodology by clinical microbiologists. As a result of this bias, when cordlike morphology is observed for NTM, these structures have been characterized as clumps, pseudocords, or loose aggregates (7, 14, 20). The resolution of the optical microscope does not allow the accurate visualization of the organization of the bacilli in these aggregates; thus, the idea that NTM cannot form true cords has persisted to the present time.

Mycobacterium abscessus is one of the most pathogenic and chemotherapy-resistant rapid-growing mycobacteria (15, 17). This mycobacterium has been reported to account for 10% of NTM lung infections in the United States and is the second most common cause of NTM lung disease in South Korea (13). *M. abscessus* causes lung disease in patients with underlying lung disorders but also in immunocompetent individuals. In addition to lung infections, this bacterium can also cause skin and soft tissue infections, meningitis, and disseminated infections (15, 17). The objective of this work was to use SEM to determine whether *M. abscessus* can also form true cords.

We have performed SEM studies of *M. abscessus* 390R, 390S, and 390V and the type strain of *M. abscessus*, DSMZ 44196^T. *Mycobacterium bovis* strain BCG Japan has been included for comparison as a member of the MTC. *M. abscessus* strain 390R was isolated in a pure culture from an ileal granuloma of a patient with Crohn's disease. This strain displays rough colonies on 7H11 agar and was reported previously to form microscopic cords by optical microscopy methods (9). Strain 390S forms smooth colonies, does not produce cords as assessed by optical microscopy, and is a spontaneous mutant of strain 390R isolated *in vitro*. In a previous work, both strains were identified as being *M. abscessus* by biochemical and genetic analyses (3). In addition to forming nonpigmented colonies and having a rapid growth rate, results of biochemical analyses showed both these variants to be *M. abscessus* (nitrate reduction negative, positive for growth in 5% NaCl, iron uptake negative, citrate negative, inositol negative, and mannitol negative). Restriction enzyme analysis was performed after PCR amplification of a portion of the gene encoding the 65-kDa heat shock protein. By using the restriction endonucleases BstEII and HaeIII, both isolates were found to be identical to the *M. abscessus* control strain (3). Strain 390V was a sponta-

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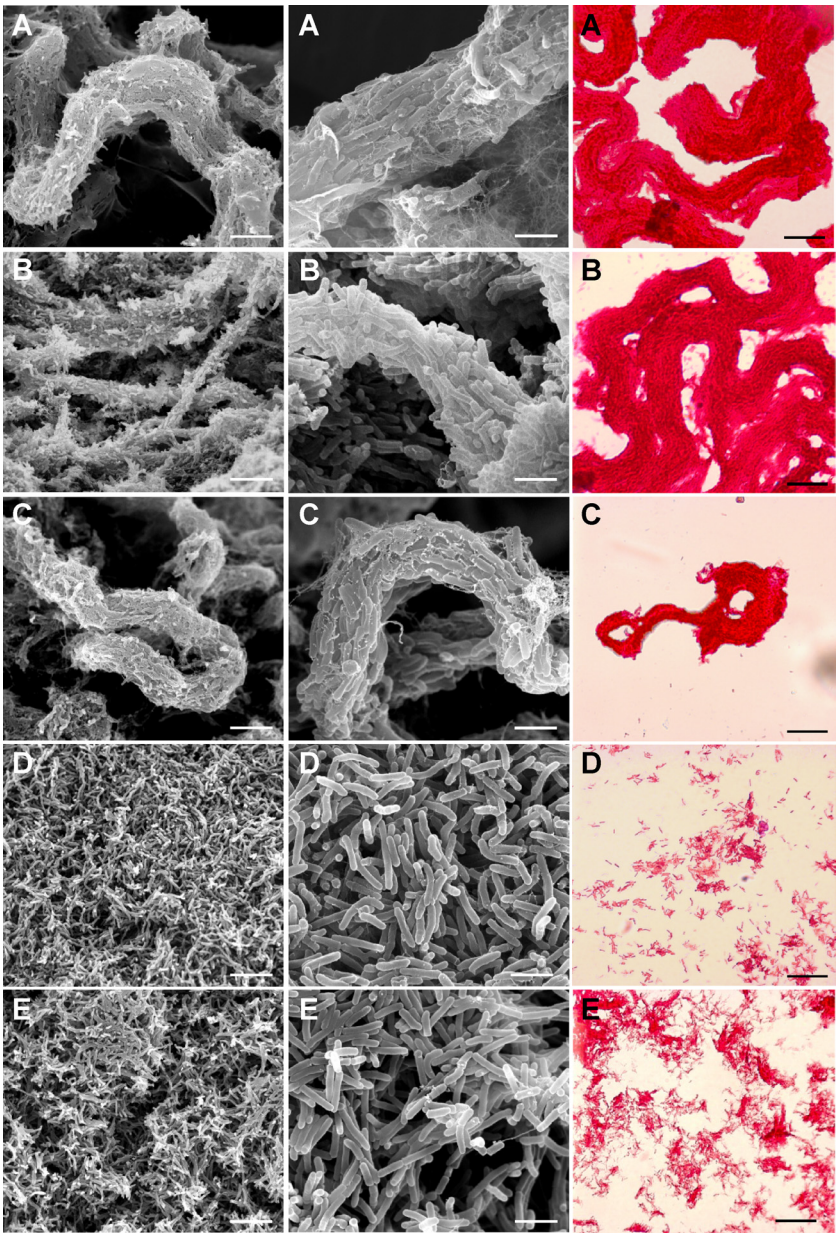

FIG. 1. Representative micrographs of SEM at medium (left) and high (middle) magnifications and photographs of Ziehl-Neelsen stains (right). Bar sizes, 7.5 μm (left), 1.9 μm (middle), and 20 μm (right). (A) *M. abscessus* 390R; (B) *M. bovis* BCG Japan; (C) *M. abscessus* 390V; (D) *M. abscessus* 390S; (E) *M. abscessus* DMSZ 44196^T.

neous rough revertant obtained during serial passages of 390S, which regained the rough-colony phenotype and the capacity to form cords, as assessed by optical microscopy (9). It was found that the smooth phenotype is due to the expression of glycopeptidolipid (GPL), which is minimally expressed by rough variants. Strains 390R and 390V are both able to replicate in human macrophages and persist in the lungs of mice, while strain 390S lacks these capabilities (9).

For all the strains studied, one isolated colony was inoculated into Trypticase soy broth (TSB) (Scharlau Chemie, Spain), and the cultures were incubated (without shaking) for 2 weeks. The cells were obtained from uncentrifuged TSB cultures by gently removing the liquid medium; that is, liquid medium was removed with a Pasteur pipette, and cells were allowed to settle to the bottom of the tube. For fixation, cells were immersed in 2.5% (vol/vol) glutaraldehyde (electron microscopy [EM] grade; Merck, Darmstadt, Germany) in 0.1 M phosphate buffer at pH 7.4 (PB; Sigma, Steinheim, Germany) for 2 h. The samples were then processed according to conventional electron microscopy methods as previously described (11). Bacilli were observed with an S-570 scanning electron microscope (Hitachi Ltd., Japan) at an accelerating voltage of 30 kV.

It is clearly shown in Fig. 1 that strain 390R forms true cords very similar to those formed by *M. bovis*. At a high magnification (also see Fig. S1 in the supplemental material), we observed the arrangement of the bacilli in the cord end to end

and side to side in parallel along the long axis of the cord. We can also appreciate in Fig. 1 and in Fig. S2 in the supplemental material that strain 390V showed cords similar to those of strain 390R and *M. bovis* strains. On the contrary, the strains that made smooth colonies, 390S and DSMZ 44196^T, did not form cords. Representative images of the Ziehl-Neelsen stains are also shown in Fig. 1. We conclude that *M. abscessus* forms true cords but that cord formation is restricted to strains forming rough colonies on solid medium.

Recent clinical reports found an association of the rough-colony phenotype with invasive infection in patients with cystic fibrosis (4, 10, 19). This association is supported by recent basic science studies that have found that in contrast to the smooth phenotype, the rough phenotype is immunostimulatory and able to replicate in human macrophages (18). It would be of interest to determine whether cord formation is a general characteristic of *M. abscessus* rough variants.

The results obtained in this study and previous studies (11, 21) demonstrate that cord formation is not specific to members of the MTC and by itself cannot identify an isolate as belonging to that complex.

Two misconceptions in the area of clinical microbiology are that only MTC organisms form microscopic cords and that the incidence of NTM is extremely low in developing countries characterized by a high burden of *M. tuberculosis* infection (8, 22). It is possible that these two misconceptions have led to misdiagnoses of multidrug-resistant tuberculosis (TB) in low-income countries where the microbiological diagnosis of TB is based only on the observation of cording morphology (5, 6, 22, 23). In support of this, recent studies reported much higher rates of isolation of NTM in Africa when rigorous mycobacterial identification techniques were applied (1, 2, 5, 8, 22). Thus, our report demonstrates that laboratories that base the microbiological identification of members of the MTC solely on the observation of microscopic cords may mistakenly identify *M. abscessus* isolates as belonging to the MTC. Since *M. abscessus* is not sensitive to drugs used to treat TB, a lack of a clinical response to therapy could lead to the erroneous conclusion that a patient is infected with multidrug-resistant TB. As a result, the patient would be exposed to an ineffective drug regimen with many adverse side effects.

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REFERENCES

1. **Bonard, D., et al.** 2004. High incidence of atypical mycobacteriosis in African HIV-infected adults with low CD4 cell counts: a 6-year cohort study in Côte d'Ivoire. AIDS **18:**1961–1964.
2. **Buijtels, P., et al.** 2009. Nontuberculous mycobacteria, Zambia. Emerg. Infect. Dis. **15:**53–55.
3. **Byrd, T. F., and C. R. Lyons.** 1999. Preliminary characterization of a *Mycobacterium abscessus* mutant in human and murine models of infection. Infect. Immun. **67:**4700–4707.
4. **Catherinot, E., et al.** 2009. Acute respiratory failure involving an R variant of *Mycobacterium abscessus*. J. Clin. Microbiol. **47:**271–274.
5. **Chihota, V. N., et al.** 2010. Liquid vs. solid culture for tuberculosis: performance and cost in a resource-constrained setting. Int. J. Tuberc. Lung Dis. **14:**1024–1031.
6. **Dos Santos Simeão, F. C., et al.** 2009. Cord factor detection and macroscopic evaluation of mycobacterial colonies: an efficient combined screening test for the presumptive identification of *Mycobacterium tuberculosis* complex on solid media. J. Bras. Pneumol. **35:**1212–1216.
7. **Glickman, M. S.** 2008. Cording, cord factors, and trehalose dimycolate, p. 63–73. *In* M. Daffé and J. M. Reyrat (ed.), The mycobacterial cell envelope. ASM Press, Washington, DC.
8. **Gopinath, K., and S. Singh.** 2010. Non-tuberculous mycobacteria in TB-endemic countries: are we neglecting the danger? PLoS Negl. Trop. Dis. **4:**e615.
9. **Howard, S. T., et al.** 2006. Spontaneous reversion of *Mycobacterium abscessus* from a smooth to a rough morphotype is associated with reduced expression of glycopeptidolipid and reacquisition of an invasive phenotype. Microbiology **152:**1581–1590.
10. **Jönsson, B. E., et al.** 2007. Molecular epidemiology of *Mycobacterium abscessus*, with focus on cystic fibrosis. J. Clin. Microbiol. **45:**1497–1504.
11. **Julián, E., et al.** 2010. Microscopic cords, a virulence-related characteristic of *Mycobacterium tuberculosis*, are also present in nonpathogenic mycobacteria. J. Bacteriol. **192:**1751–1760.
12. **Kaminski, D. A., and D. J. Hardy.** 1995. Selective utilization of DNA probes for identification of *Mycobacterium* species on the basis of cord formation in primary BACTEC 12B cultures. J. Clin. Microbiol. **33:**1548–1550.
13. **Koh, W.-J., et al.** 2006. Clinical significance of nontuberculous mycobacteria isolated from respiratory specimens in Korea. Chest **129:**341–348.
14. **McCarter, Y. S., I. N. Ratkiewicz, and A. Robinson.** 1998. Cord formation in Bactec medium is a reliable, rapid method for presumptive identification of *Mycobacterium tuberculosis* complex. J. Clin. Microbiol. **36:**2769–2771.
15. **Medjahed, H., J.-L. Gaillard, and J.-M. Reyrat.** 2010. *Mycobacterium abscessus*: a new player in the mycobacterial field. Trends Microbiol. **18:**117–123.
16. **Middlebrook, G., R. J. Dobos, and C. Pierce.** 1947. Virulence and morphological characteristics of mammalian tubercle bacilli. J. Exp. Med. **861:**175–184.
17. **Petrini, B.** 2006. *Mycobacterium abscessus*: an emerging rapid-growing potential pathogen. APMIS **114:**319–328.
18. **Rhoades, E. R., et al.** 2009. *Mycobacterium abscessus* glycopeptidolipid masks underlying cell wall phosphatidyl-*myo*-inositol mannosides preventing their interaction with human monocyte TLR2. J. Immunol. **183:**1997–2007.
19. **Sanguinetti, M., et al.** 2001. Fatal pulmonary infection due to multidrug-resistant *Mycobacterium abscessus* in a patient with cystic fibrosis. J. Clin. Microbiol. **39:**816–819.
20. **Shen, G. H., et al.** 2009. Combining the Capilia TB assay with smear morphology for the identification of *Mycobacterium tuberculosis* complex. Int. J. Tuberc. Lung Dis. **13:**371–376.
21. **Staropoli, J. F., and J. A. Branda.** 2008. Cord formation in a clinical isolate of *Mycobacterium marinum*. J. Clin. Microbiol. **46:**2814–2816.
22. **Tortoli, E., et al.** 2009. Infection due to a novel mycobacterium, mimicking multidrug-resistant *Mycobacterium tuberculosis*. Clin. Microbiol. Infect. **16:**1130–1134.
23. **Urbanczik, R.** 2010. Laboratory tests focusing on sputum. Int. J. Tuberc. Lung Dis. **14:**1087–1093.